

We Claim:

1. A subunit vaccine composition comprising one or more isolated CAL virus immunogens and a pharmaceutically acceptable vehicle, wherein the one or more immunogens are selected from the group consisting of (a) G1, (b) G2, (c) N, (d) NSm, (e) NSs, (f); immunogenic fragments of (b), (c), (d) or (e); and immunogenic analogs of (a), (b), (c), (d), (e) or (f).
2. The subunit vaccine composition of claim 1, comprising an immunogen with the sequence of amino acids depicted at positions 474-1441 of Figures 1A-1E, or a sequence of amino acids with at least 75% sequence identity thereto.
3. The subunit vaccine composition of claim 2, wherein the immunogen comprises a sequence of amino acids with at least 85% sequence identity to the sequence of amino acids depicted at positions 474-1441 of Figures 1A-1E.
4. The subunit vaccine composition of claim 2, wherein the immunogen comprises a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids depicted at positions 474-1441 of Figures 1A-1E.
5. The subunit vaccine composition of claim 2, wherein the immunogen comprises the sequence of amino acids depicted at positions 474-1441 of Figures 1A-1E.
6. The subunit vaccine composition of claim 1, wherein the immunogen comprises the sequence of amino acids depicted at positions 1-1441 of Figures 1A-1E, or a sequence of amino acids with at least 75% sequence identity thereto.
7. The subunit vaccine composition of claim 6, wherein the immunogen comprises a sequence of amino acids with at least 85% sequence identity to the sequence of amino acids depicted at positions 1-1441 of Figures 1A-1E.

8. The subunit vaccine composition of claim 6, wherein the immunogen comprises a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids depicted at positions 1-1441 of Figures 1A-1E.

5 9. The subunit vaccine composition of claim 6, wherein the immunogen comprises the sequence of amino acids depicted at positions 1-1441 of Figures 1A-1E.

10 10. The subunit vaccine composition of claim 7, comprising an immunogenic fusion polypeptide that comprises a LACV envelope polypeptide fused to at least one other CAL virus polypeptide.

15 11. An immunogenic composition comprising a CAL virus truncated G1 polypeptide, wherein the truncated G1 polypeptide is truncated at a position between amino acid position 1391 and the C-terminus of the native G1 envelope polypeptide, numbered relative to the G1 polypeptide depicted in Figures 1A-1E.

20 12. The immunogenic composition of claim 11, wherein the truncated G1 polypeptide comprises the sequence of amino acids depicted at amino acid positions 474-1391 of Figures 1A-1E.

13. An immunogenic composition comprising at least one isolated CAL virus immunogen, wherein said immunogen is produced intracellularly.

25 14. The immunogenic composition of claim 13, wherein said immunogen is one or more immunogens selected from the group consisting of (a) G1, (b) G2, (c) N, (d) NSm, (e) NSs, (f); immunogenic fragments of (a), (b), (c), (d) or (e); and immunogenic analogs of (a), (b), (c), (d), (e) or (f).

30 15. The immunogenic composition of claim 14, comprising a full-length G1.

16. The immunogenic composition of claim 14, comprising a truncated G1 polypeptide.

17. The immunogenic composition of claim 16, wherein the truncated G1 polypeptide comprises a deletion of all or part of a transmembrane binding domain.

18. The immunogenic composition of claim 17, wherein the truncated G1
5 polypeptide further comprises a deletion of all or part of the cytoplasmic tail.

19. The immunogenic composition of claim 17, wherein the truncated G1 polypeptide comprises all or part of the cytoplasmic tail.

10 20. The immunogenic composition of claim 17, wherein the truncated G1 polypeptide is truncated at a position between amino acid position 1387 and the C-terminus of the native G1 envelope polypeptide, numbered relative to the G1 polypeptide depicted in Figures 1A-1E.

15 21. The immunogenic composition of claim 17, wherein the truncated G1 polypeptide is truncated at a position between amino acid position 1391 and the C-terminus of the native G1 envelope polypeptide, numbered relative to the G1 polypeptide depicted in Figures 1A-1E.

20 22. The immunogenic composition of claim 20, wherein the truncated G1 polypeptide comprises the sequence of amino acids depicted at amino acid positions 474 to 1387 of Figures 1A-1E.

25 23. The immunogenic composition of claim 21, wherein the truncated G1 polypeptide comprises the sequence of amino acids depicted at amino acid positions 474-1391 of Figures 1A-1E.

30 24. The immunogenic composition of claim 22, wherein the truncated G1 polypeptide comprises a deletion of amino acids 1388-1419, numbered relative to the G1 polypeptide depicted in Figures 1A-1E.

25. The immunogenic composition of claim 23, wherein the truncated G1 polypeptide comprises a deletion of amino acids 1392-1419, numbered relative to the G1 polypeptide depicted in Figures 1A-1E.

26. The immunogenic composition of claim 13, comprising the protein product of a CAL virus M region.

5 27. The immunogenic composition of claim 15, comprising the sequence of amino acids depicted at positions 474-1441 of Figures 1A-1E, or a sequence of amino acids with at least 75% sequence identity thereto.

10 28. The immunogenic composition of claim 27, comprising a sequence of amino acids with at least 85% sequence identity to the sequence of amino acids depicted at positions 474-1441 of Figures 1A-1E.

15 29. The immunogenic composition of claim 27, comprising a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids depicted at positions 474-1441 of Figures 1A-1E.

30. The immunogenic composition of claim 27, comprising the sequence of amino acids depicted at positions 474-1441 of Figures 1A-1E.

20 31. The immunogenic composition of claim 26, comprising the sequence of amino acids depicted at positions 1-1441 of Figures 1A-1E, or a sequence of amino acids with at least 75% sequence identity thereto.

25 32. The immunogenic composition of claim 31, comprising a sequence of amino acids with at least 85% sequence identity to the sequence of amino acids depicted at positions 1-1441 of Figures 1A-1E.

30 33. The immunogenic composition of claim 31, comprising a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids depicted at positions 1-1441 of Figures 1A-1E.

34. The immunogenic composition of claim 31, comprising the sequence of amino acids depicted at positions 1-1441 of Figures 1A-1E.

35. An immunogenic composition comprising an inactivated CAL virus and a pharmaceutically acceptable vehicle.
36. The immunogenic composition of claim 1, wherein the CAL virus is La Crosse virus.
37. An immunogenic composition comprising an attenuated CAL virus and a pharmaceutically acceptable vehicle.
38. The immunogenic composition of claim 3, wherein the CAL virus is La Crosse virus.
39. A method of treating or preventing CAL virus infection in a mammalian subject comprising administering to said subject a therapeutically effective amount of the immunogenic composition of claim 1.
40. A method of treating or preventing CAL virus infection in a mammalian subject comprising administering to said subject a therapeutically effective amount of the immunogenic composition of claim 11.
41. A method of treating or preventing CAL virus infection in a mammalian subject comprising administering to said subject a therapeutically effective amount of the immunogenic composition of claim 13.
42. A method of treating or preventing CAL virus infection in a mammalian subject comprising administering to said subject a therapeutically effective amount of the immunogenic composition of claim 35.
43. A method of treating or preventing CAL virus infection in a mammalian subject comprising administering to said subject a therapeutically effective amount of the subunit vaccine composition of claim 36.

44. A method of treating or preventing CAL virus infection in a mammalian subject comprising administering to said subject a therapeutically effective amount of the immunogenic composition of claim 37.

5 45. A method of producing an immunogenic composition comprising the steps of

- (a) providing an inactivated or attenuated CAL virus; and
- (b) combining said inactivated or attenuated CAL virus with a pharmaceutically acceptable vehicle.

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46. A method of producing a subunit vaccine composition comprising the steps of

- (a) providing one or more CAL virus immunogens, wherein the one or more immunogens are selected from the group consisting of (a) G1, (b) G2, (c) N, (d) NSm, (e) NSs, (f); immunogenic fragments of (b), (c), (d) or (e); and immunogenic analogs of (a), (b), (c), (d), (e) or (f); and
- (b) combining said CAL virus immunogen(s) with a pharmaceutically acceptable vehicle.

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47. A method of producing an immunogenic composition comprising the steps of

- (a) providing a CAL virus immunogen, wherein said immunogen is produced intracellularly
- (b) combining said CAL virus immunogen with a pharmaceutically acceptable vehicle.

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48. A method of producing an immunogenic composition comprising the steps of

- (a) providing a CAL virus truncated G1 polypeptide, wherein the truncated G1 polypeptide is truncated at a position between amino acid position 1391 and the C-terminus of the native G1 envelope polypeptide, numbered relative to the G1 polypeptide depicted in Figures 1A-1E; and
- (b) combining said CAL virus truncated G1 polypeptide with a pharmaceutically acceptable vehicle.

49. A method for isolating an immunogenic CAL virus envelope polypeptide comprising:

- (a) providing a population of mammalian host cells that express said envelope polypeptide intracellularly;
- 5 (b) recovering a membrane component of the cells;
- (c) treating the membrane component with a non-ionic detergent, thereby to solubilize the membrane component and release the envelope polypeptide; and
- (d) isolating the released envelope polypeptide.

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50. The method of claim 49, wherein said isolating comprises at least one column purification step wherein said column is selected from the group consisting of a lectin affinity column, a hydroxyapatite column and an ion exchange column.

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51. The method of claim 50, wherein said isolating step comprises:

- (i) binding the released envelope polypeptide to the ion exchange column; and
- (ii) eluting the bound envelope polypeptide from the ion exchange column.

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52. The method of claim 50, wherein said isolating step comprises:

- (i) binding the released envelope polypeptide to a lectin affinity column;
- (ii) eluting the bound polypeptide from the lectin affinity column;
- (iii) subjecting the eluted polypeptide to a cation exchange column; and
- (iv) eluting the bound envelope polypeptide from the cation exchange column.

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53. The method of claim 52, where said lectin affinity column is a

concanavalin A lectin column.

54. The method of claim 50, wherein the mammalian cells are CHO or HEK293 cells.

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55. The method of claim 49, wherein the CAL virus envelope polypeptide is a G1 and/or a G2 polypeptide, and optionally includes all or a portion of the NSm polypeptide.

56. An immunogenic composition comprising the envelope polypeptide obtained by the method of claim 49.

5 57. A CAL virus truncated G1 polypeptide, wherein the truncated G1 polypeptide is truncated at a position between amino acid position 1391 and the C-terminus of the native G1 envelope polypeptide, numbered relative to the G1 polypeptide depicted in Figures 1A-1E.

10 58. The truncated G1 polypeptide of claim 57, wherein the polypeptide comprises the sequence of amino acids depicted at amino acid positions 474-1391 of Figures 1A-1E.

15 59. An isolated oligonucleotide not more than 60 nucleotides in length comprising:

- (a) a nucleotide sequence of at least 10 contiguous nucleotides from a probe or primer sequence depicted in any of Figures 5, 6 or 7;
- (b) a nucleotide sequence having 90% sequence identity to a nucleotide sequence of (a); or
- 20 (c) complements of (a) and (b).

60. The oligonucleotide of claim 59, wherein the nucleotide sequence is a probe sequence depicted in any of Figures 5, 6 or 7 and further comprises a detectable label at the 5'-end and/or the 3'-end.

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61. The oligonucleotide of claim 60, wherein the detectable label is a fluorescent label selected from the group consisting of 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7', - tetrachloro -4-7-dichlorofluorescein (TET).

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62. An isolated oligonucleotide selected from the group consisting of: (a) the oligonucleotide of SEQ ID NO:7, (b) the oligonucleotide of SEQ ID NO:8, (c) the oligonucleotide of SEQ ID NO:9, (d) the oligonucleotide of SEQ ID NO:10, (e) the oligonucleotide of SEQ ID NO:11, (f) the oligonucleotide of SEQ ID NO:12, (g) the

oligonucleotide of SEQ ID NO:13, (h) the oligonucleotide of SEQ ID NO:14, (i) the oligonucleotide of SEQ ID NO:15, (j) SEQ ID NO:16, complements of (a), (b), (c), (d), (e), (f), (g), (h), (i) or (j), and reverse complements of (a), (b), (c), (d), (e), (f), (g), (h), (i) or (j).

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63. The oligonucleotide of claim 62, wherein said oligonucleotide is selected from the group consisting of (a) the oligonucleotide of SEQ ID NO:8, (b) the oligonucleotide of SEQ ID NO:9, (c) the oligonucleotide of SEQ ID NO:12, (d) the oligonucleotide of SEQ ID NO:16, complements of (a), (b), (c) or (d), and reverse complements of (a), (b), (c) or (d).

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64. The oligonucleotide of claim 62, comprising a detectable label at the 5'-end and/or the 3'-end.

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65. The oligonucleotide of claim 63, comprising a detectable label at the 5'-end and/or the 3'-end.

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66. The oligonucleotide of claim 64, wherein the detectable label is a fluorescent label selected from the group consisting of 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7', - tetrachloro -4-7-dichlorofluorescein (TET).

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67. The oligonucleotide of claim 65, wherein the detectable label is a fluorescent label selected from the group consisting of 6-carboxyfluorescein (6-FAM), tetramethyl rhodamine (TAMRA), and 2', 4', 5', 7', - tetrachloro -4-7-dichlorofluorescein (TET).

68. A method for detecting CAL virus infection in a biological sample, the method comprising:

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(a) isolating nucleic acid from a biological sample suspected of containing CAL virus RNA, wherein if CAL virus is present, said nucleic acid comprises a target sequence;

(b) reacting the CAL virus nucleic acid with a detectably labeled probe sufficiently complementary to and capable of hybridizing with the target sequence,

wherein said reacting is done under conditions that provide for the formation of a probe/target sequence complex; and

(c) detecting the presence or absence of label as an indication of the presence or absence of the target sequence.

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69. A method for detecting La Crosse virus (LACV) infection in a biological sample, the method comprising:

(a) isolating nucleic acid from a biological sample suspected of containing LACV RNA, wherein if LACV is present, said nucleic acid comprises a target

10 sequence;

(b) reacting the LACV nucleic acid with a detectably labeled probe sufficiently complementary to and capable of selectively hybridizing with the target sequence, wherein said reacting is done under conditions that provide for the formation of a probe/target sequence complex; and

15 (c) detecting the presence or absence of label as an indication of the presence or absence of the target sequence.

70. The method of claim 69, wherein the probe is selected from the group consisting of (a) the oligonucleotide of SEQ ID NO:8, (b) the oligonucleotide of SEQ 20 ID NO:9, (c) the oligonucleotide of SEQ ID NO:12, (d) the oligonucleotide of SEQ ID NO:16, complements of (a), (b), (c) or (d), and reverse complements of (a), (b), (c) or (d).

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71. A method for detecting CAL virus infection in a biological sample, the method comprising:

isolating nucleic acids from a biological sample suspected of containing CAL virus;

amplifying the nucleic acids using at least two primers wherein (a) each of the primers is not more than about 50 nucleotides in length and each of the primers is sufficiently complementary to a portion of the sense and antisense strands, respectively, of CAL virus isolated nucleic acid, if present, to hybridize therewith; 30 and

detecting the presence of the amplified nucleic acids as an indication of the presence or absence of CAL virus in the sample.

72. The method of claim 71, wherein amplifying comprises RT-PCR, transcription-mediated amplification (TMA) or a fluorogenic 5' nuclease assay, or a combination thereof.

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73. The method of claim 72, wherein amplifying uses a fluorogenic 5' nuclease assay using the sense primer and the antisense primer and detecting is done using at least one detectably labeled probe sufficiently complementary to and capable of hybridizing with the CAL virus nucleic acid if present.

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74. A method for detecting La Crosse virus (LACV) infection in a biological sample, the method comprising:

isolating nucleic acids from a biological sample suspected of containing LACV wherein if LACV is present, said nucleic acid comprises a target sequence;

15 amplifying the nucleic acids using at least two primers wherein (a) each of the primers is not more than about 50 nucleotides in length and each of the primers is sufficiently complementary to a portion of the sense and antisense strands, respectively, of LACV isolated nucleic acid, if present, to hybridize therewith, and further wherein at least one of the primers is capable of selectively hybridizing to the

20 target sequence; and

detecting the presence of the amplified nucleic acids as an indication of the presence or absence of LACV in the sample.

75. The method of claim 74, wherein amplifying comprises RT-PCR, transcription-mediated amplification (TMA) or a fluorogenic 5' nuclease assay, or a combination thereof.

76. The method of claim 75, wherein amplifying uses a fluorogenic 5' nuclease assay using the sense primer and the antisense primer and detecting is done using at least one detectably labeled probe sufficiently complementary to and capable of hybridizing with the LACV nucleic acid if present.

77. The method of claim 74, wherein one of the primers is selected from the group consisting of (a) the oligonucleotide of SEQ ID NO:8, (b) the oligonucleotide

of SEQ ID NO:9, (c) the oligonucleotide of SEQ ID NO:12, (d) the oligonucleotide of SEQ ID NO:16, complements of (a), (b), (c) or (d), and reverse complements of (a), (b), (c) or (d).

5 78. A method for detecting La Crosse virus (LACV) infection in a biological sample, the method comprising:

isolating nucleic acids from a biological sample suspected of containing LACV wherein if LACV is present, said nucleic acid comprises a target sequence; amplifying the nucleic acids using at least two primers wherein (a) each of the 10 primers is not more than about 50 nucleotides in length and each of the primers is sufficiently complementary to a portion of the sense and antisense strands, respectively, of LACV isolated nucleic acid, if present, to hybridize therewith; and detecting the presence of the amplified nucleic acids using at least one detectably labeled probe sufficiently complementary to and capable of hybridizing 15 with the LACV nucleic acid if present, as an indication of the presence or absence of LACV in the sample, wherein at least one of the primers and/or the probe is capable of selectively hybridizing to the target sequence.

20 79. The method of claim 78, wherein one of the primers is selected from the group consisting of (a) the oligonucleotide of SEQ ID NO:8, (b) the oligonucleotide of SEQ ID NO:9, (c) the oligonucleotide of SEQ ID NO:12, (d) the oligonucleotide of SEQ ID NO:16, complements of (a), (b), (c) or (d), and reverse complements of (a), (b), (c) or (d).

25 80. A kit for detecting a CAL virus infection in a biological sample, the kit comprising:

primer oligonucleotides wherein the primer oligonucleotides are not more than about 60 nucleotides in length, wherein each of the primers is sufficiently complementary to a portion of the sense and antisense strands, respectively, to CAL 30 virus nucleic acid to hybridize therewith; and written instructions for identifying the presence of a CAL virus.

81. The kit of claim 66, further comprising a polymerase and buffers.

82. The kit of claim 66, further comprising at least one detectably labeled probe oligonucleotide of not more than about 60 nucleotides in length and sufficiently complementary to and capable of hybridizing with CAL virus nucleic acid.

5 83. A kit for detecting a La Crosse virus (LACV) infection in a biological sample, the kit comprising:

primer oligonucleotides wherein the primer oligonucleotides are not more than about 60 nucleotides in length, wherein each of the primers is sufficiently complementary to a portion of the sense and antisense strands, respectively, to LACV
10 nucleic acid to hybridize therewith and further wherein at least one of the primers is capable of selectively hybridizing to LACV nucleic acid; and
written instructions for identifying the presence of a LACV.

15 84. The kit of claim 83, further comprising a polymerase and buffers.

15 85. The kit of claim 83, wherein one of the primers is selected from the group consisting of (a) the oligonucleotide of SEQ ID NO:8, (b) the oligonucleotide of SEQ ID NO:9, (c) the oligonucleotide of SEQ ID NO:12, (d) the oligonucleotide of SEQ ID NO:16, complements of (a), (b), (c) or (d), and reverse complements of (a), (b), (c) or (d).

20 86. The kit of claim 83, further comprising at least one detectably labeled probe oligonucleotide of not more than about 60 nucleotides in length and sufficiently complementary to and capable of hybridizing with LACV nucleic acid.

25 87. A kit for detecting a La Crosse virus (LACV) infection in a biological sample, the kit comprising:

primer oligonucleotides wherein the primer oligonucleotides are not more than about 60 nucleotides in length, wherein each of the primers is sufficiently complementary to a portion of the sense and antisense strands, respectively, to LACV nucleic acid to hybridize therewith;

at least one detectably labeled probe oligonucleotide of not more than about 60 nucleotides in length and sufficiently complementary to and capable of hybridizing

with LACV nucleic acid, wherein at least one of the primers and/or the probe is capable of selectively hybridizing to the target sequence; and
written instructions for identifying the presence of LACV.

5 88. The kit of claim 87, further comprising a polymerase and buffers.

89. The kit of claim 87, wherein one of the primers and/or probes is selected from the group consisting of (a) the oligonucleotide of SEQ ID NO:8, (b) the oligonucleotide of SEQ ID NO:9, (c) the oligonucleotide of SEQ ID NO:12, (d) the oligonucleotide of SEQ ID NO:16, complements of (a), (b), (c) or (d), and reverse complements of (a), (b), (c) or (d).

10 90. The subunit vaccine composition of claim 1, wherein the immunogen is produced by recombinant expression of a polynucleotide encoding a polypeptide with 15 the sequence of amino acids depicted at positions 474-1441 of Figures .

91. The subunit vaccine composition of claim 1, wherein the immunogen is produced by recombinant expression of a polynucleotide encoding a polypeptide with the sequence of amino acids depicted at positions 474-1441 of Figures 1A-1E.

20 92. The subunit vaccine composition of claim 1, wherein the immunogen is produced by recombinant expression of a polynucleotide encoding a polypeptide with the sequence of amino acids depicted at positions 1-1441 of Figures 1A-1E.

25 93. The immunogenic composition of claim 11, wherein the truncated G1 polypeptide is produced by recombinant expression of a polynucleotide encoding a polypeptide with the sequence of amino acids depicted at positions 474-1391 of Figures 1A-1E.

30 94. The immunogenic composition of claim 13, wherein the immunogen is produced by recombinant expression of a polynucleotide encoding a polypeptide with the sequence of amino acids depicted at positions 474-1441 of Figures 1A-1E.

95. The immunogenic composition of claim 13, wherein the immunogen is produced by recombinant expression of a polynucleotide encoding a polypeptide with the sequence of amino acids depicted at positions 1-1441 of Figures 1A-1E.

5 96. The immunogenic composition of claim 13, wherein the immunogen is produced by recombinant expression of a polynucleotide encoding a polypeptide with the sequence of amino acids depicted at positions 474-1391 of Figures 1A-1E.

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